SUPPLEMENTARY INFORMATION

Heat-triggered remote control of CRISPR-dCas9 for tunable transcriptional modulation

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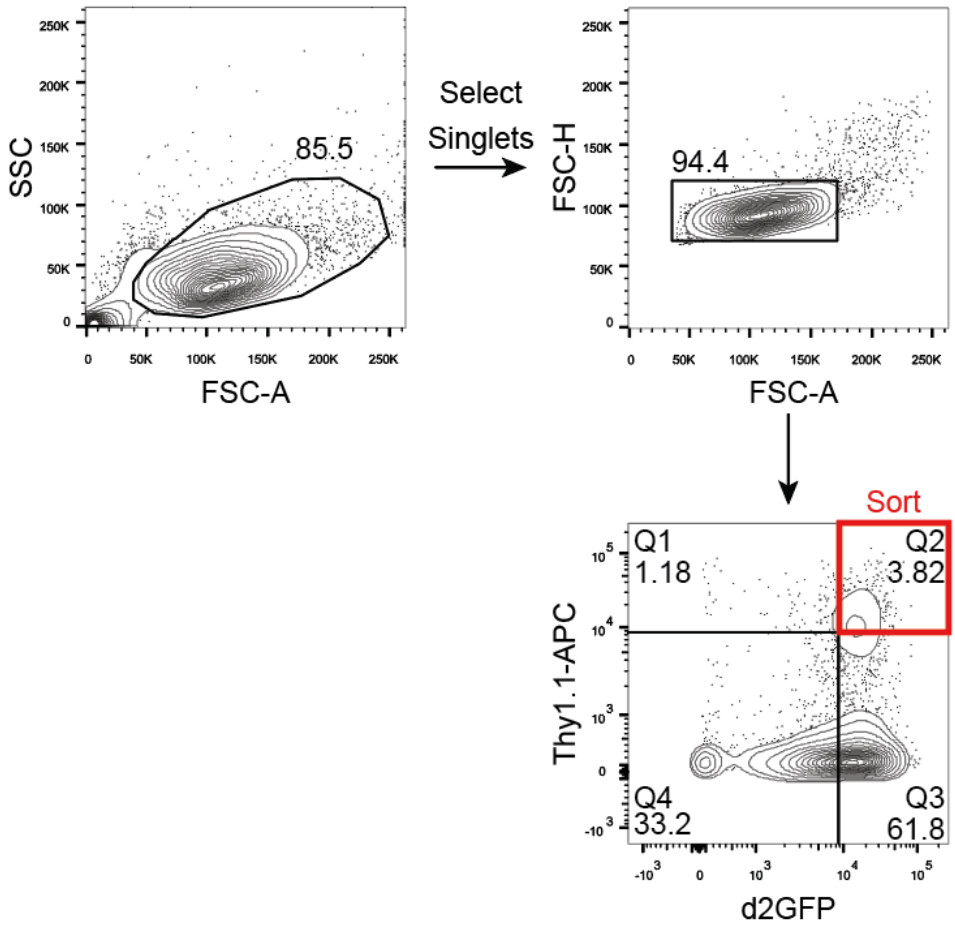
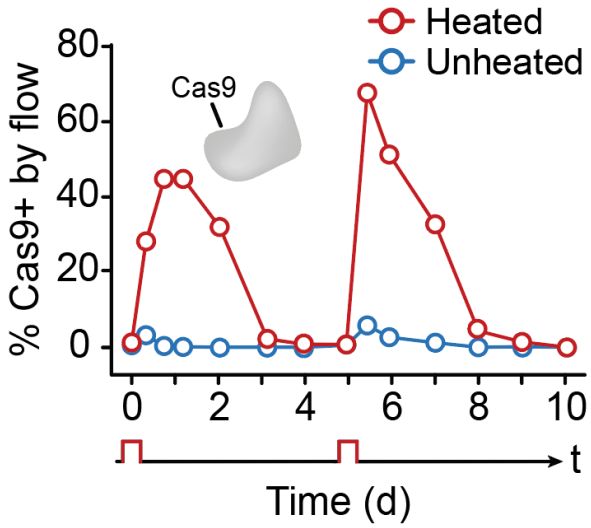
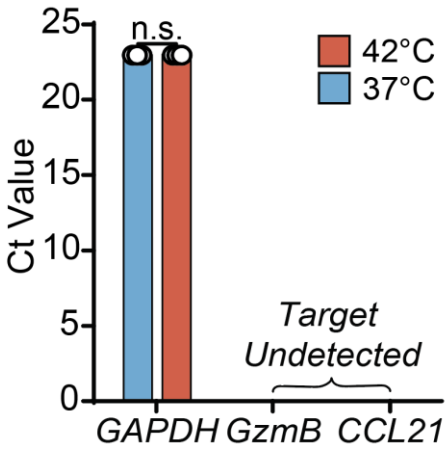
SUPPLEMENTARY RESULTS



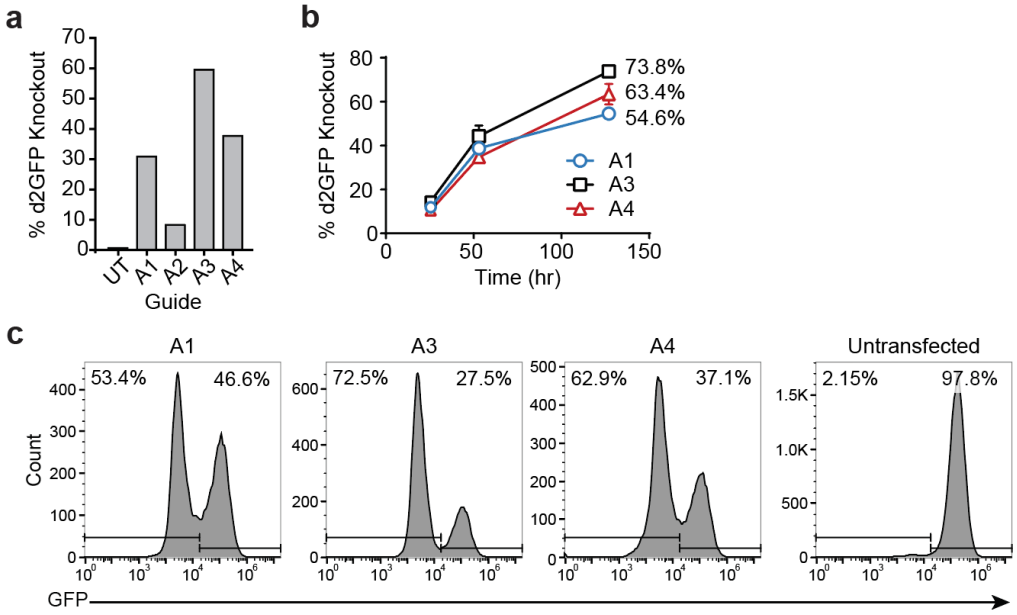
Supplementary Figure 1 | Gating strategy for selecting HEK293T cells for d2GFP suppression . Gating strategy used for selecting cells that are double positive for the thermal switch (reporter: Thy1.1) and sgRNAs (reporter: d2GFP).



Supplementary Figure 2 | Heat-triggered gene switch enables control of Cas9 expression. Kinetic trace of Cas9 expression following 30 min of heating at 42°C treated at 0 and 4 or 5 d (n=3, mean ± s.d., blue trace=37°C, red trace = 42°C).



Supplementary Figure 3 | *GAPDH*, *GzmB*, and *CCL21* transcript levels in WT cells assayed 72 hrs post heating once for 30 min at 42°C (n = 4, mean ± s.d., unpaired t-test, *p<0.05).



Supplementary Figure 4 | Scouting sgRNAs for d2GFP Suppression. eSpCas9(1.1) plasmids containing sgRNA sequences targeting the first 100 bps following the translational start site (Supplementary Table 2) were transfected into d2GFP-expressing HEK293T cells. (a) d2GFP knockout was measured by flow cytometry 9 days post transfection. (b) The kinetics of d2GFP knockout were measured on the top performing guides (*n=3*, mean ± s.d.). (c) Representative flow plots of d2GFP expression 127 hrs post transfection.

Supplementary Table 1 | sgRNA sequences for transcriptional activation.

Target Gene	Strand	Guide #	Sequence (5'-3')
CCL21	Top	1	CACCGGGTAGCTGGGAATAGAAGGA
		2	CACCGGAGGGGAAGGGTATGGATCC
		3	CACCGGAGACAGTCATGGTGTTCCA
		4	CACCGGACATAAAATTTGGCAGCTG
		5	CACCGGCGTAGTGAGGAGACAGTCA
	Bottom	1	AAACTCCTTCTATTCCCAGCTACCC
		2	AAACGGATCCATACCCTTCCCCTCC
		3	AAACTGGAACACCATGACTGTCTCC
		4	AAACCAGCTGCCAAATTTTATGTCC
		5	AAACTGACTGTCTCCTCACTACGCC
GZMB	Top	1	CACCGGGCACCCAGAGGACGTCATC
		2	CACCGGAGAGGACGTCATCAGGCAG
		3	CACCGGTCAGCTGTGGGTGATGATG
		4	CACCGGACTCTGAGTCATCAGCTGT
		5	CACCGGCTGCTCTGGGCTGAATAGG
	Bottom	1	AAACGATGACGTCCTCTGGGTGCCC
		2	AAACCTGCCTGATGACGTCCTCTCC
		3	AAACCATCATCACCCACAGCTGACC
		4	AAACACAGCTGATGACTCAGAGTCC
		5	AAACCCTATTCAGCCCAGAGCAGCC

Supplementary Table 2 | sgRNA sequences for d2GFP suppression.

Target Gene	Strand	Guide ID	Sequence (5'-3')
d2GFP	Top	A1	CACCGCGAGGAGCTGTTCACCGGGG
		A2	CACCGCACCGGGGTGGTGCCCATCC
		A3	CACCGGACCAGGATGGGCACCACCC
		A4	CACCGAAGGGCGAGGAGCTGTTCAC
	Bottom	A1	AAACCCCCGGTGAACAGCTCCTCGC
		A2	AAACGGATGGGCACCACCCCGGTGC
		A3	AAACGGGTGGTGCCCATCCTGGTCC
		A4	AAACGTGAACAGCTCCTCGCCCTTC